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Butyrate and deoxycholic acid play common and distinct roles in HCT116 human colon cell proliferation☆,☆☆,★

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Abstract

Consumption of a high-fat diet causes an increase in bile acid deoxycholic acid (DCA) in colon lumen and colon cancer risk, while butyrate, an intestinal microbiota metabolite of dietary fiber, has been shown to exhibit colon cancer-preventive effects. To distinguish these opposing effects of DCA and butyrate (two major metabolites in colon lumen), we examined the effects of physiologically relevant doses of butyrate (0.5–2 mmol/l) and DCA (0.05–0.3 mmol/l) on colon cell proliferation. We hypothesize that butyrate and DCA each modulates the cell cycle and apoptosis via common and distinct cellular signaling targets. In this study, we demonstrated that both butyrate and DCA inhibited cell proliferation by up to 89% and 92% and increased cell apoptosis rate by up to 3.1- and 4.5-fold, respectively. Cell cycle analyses revealed that butyrate led to an increase in G1 and G2 fractions with a concomitant drop in the S-phase fraction, but DCA induced an increase in only G1 fraction with a concomitant drop in the S-phase fraction when compared with the untreated cells. The examination of early cellular signaling revealed that DCA but not butyrate increased intracellular reactive oxygen species, genomic DNA breakage, the activation of ERK1/2, caspase-3 and PARP. In contrast, DCA decreased activated Rb protein level, and butyrate but not DCA increased p21 expression. Collectively, although both butyrate and DCA inhibit colonic cell proliferation, butyrate increases tumor suppressor gene expression, whereas DCA decreases tumor suppressor activation in cell cycle and apoptosis pathways.
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Keywords: Apoptosis; Butyrate; Colon cancer; Cell cycle; Deoxycholic acid

1. Introduction

Colon cancer accounts for approximately 140,000 new cancer cases and 50,000 deaths each year in the United States, and it is predicted that half the Western population will develop at least one colorectal tumor by the age of 70 years [1]. It has been reported that consuming high levels of dietary fiber or resistant starches have a lower risk of colon cancer in human populations and animal models [2,3]. This effect may be related to butyrate, the short-chain fatty acid (SCFA), production in the colonic lumen by the bacterial fermentation of dietary fiber [2–4]. The colonic luminal SCFA concentration could reach 10 mmol/l when humans consume moderate fiber diets [5,6]. Conceivably, there is a continuous butyrate exposure in the colonic epithelium, and butyrate may exert several anticarcinogenic effects through the modulation of the colon cell cycle and apoptosis [7–9]. In contrast, it has been shown that bile acid concentration could reach 1 mmol/l in the colon after the consumption of a high-fat meal [10,11], and these bile acids, primarily deoxycholic acid (DCA) in humans, are believed to be tumor promoters of colon cancer [12,13]. For example, there is an increased frequency of apoptosis at colon crypts only during the first 15 weeks when mice were fed with a Western-type diet [10]. Interestingly, these mice fed with a high-fat diet no longer had greatly increased levels of apoptosis but had much higher frequencies of atypical nuclei at later ages, which would result in cancer development [10,14]. Several lines of evidence have suggested that cell growth inhibition induced by DCA may cause compensatory hyperproliferation of a subpopulation of colonic epithelial cells resistant to DCA's inhibitory effect, and ultimately alters colon cell proliferation [15]. Consistent with these observations, a recent human study demonstrated a strong association between low colonic butyrate and high DCA in populations with high colon cancer risk [16].

In viewing the fact that both butyrate and DCA inhibit colon cell proliferation at physiological concentrations, although their opposing effects on colon carcinogenetic process [7,8,12,15], we hypothesize that butyrate inhibits colon cancer cell proliferation through a mechanism that is different from the one used by DCA, which may

Abbreviations: Bid, BH3-interacting domain; DCA, deoxycholic acid; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; ERK1/2, extracellular-regulated kinase 1/2; NaB, sodium butyrate; PARP, poly-ADP ribose polymerase; Rb, retinoblastoma protein.

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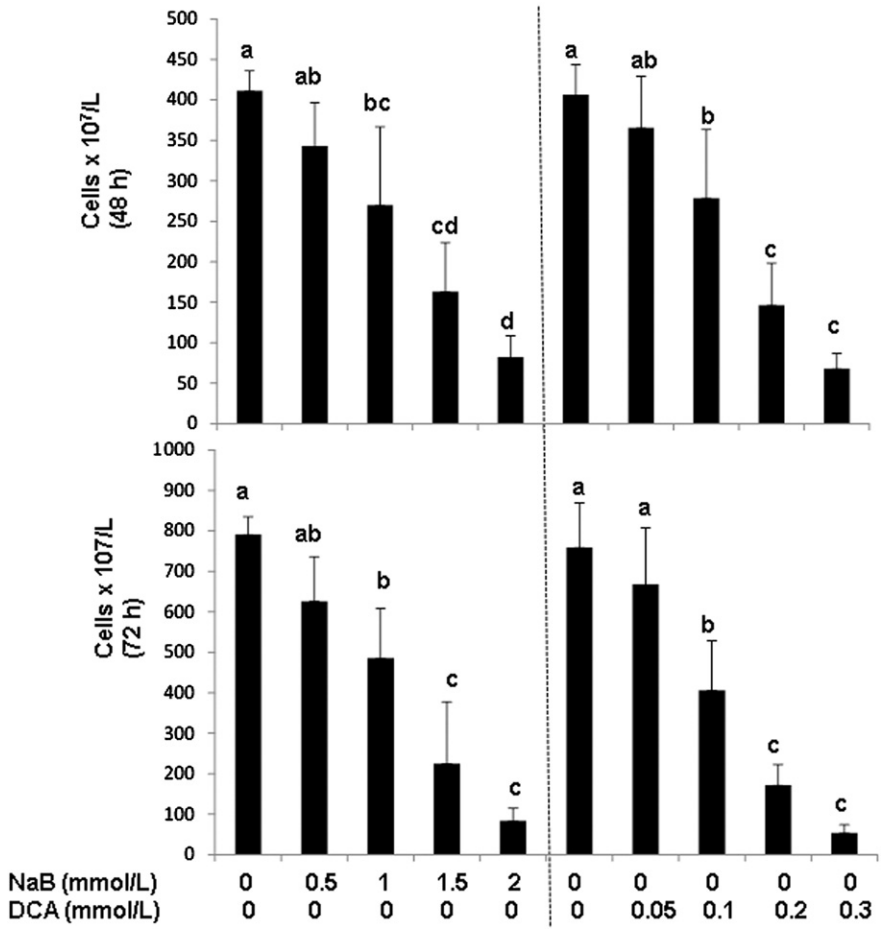

Fig. 1. Effect of NaB and DCA on the growth of HCT116 colon cells for 48 and 72 h. Values are means±S.D., n=4. Means at a time without a common letter differ, P<.05.

be part of the molecular basis for the opposing effects of butyrate and DCA on colon tumorigenesis. Therefore, it is important to determine the common and different signaling pathways by which butyrate and DCA modulate colon cancer cell proliferation in the same colon cell line.

The colon has a flat surface epithelium composed of colonocytes, enteroendocrine cells and goblet cells invaginating to form crypts. Crypt cells divide rapidly and travel to the top of the epithelium where they differentiate, proliferate and undergo cell cycle progress and apoptosis within 48–72 h [17]. HCT116, a colonic epithelial cell line (sensitive to apoptosis), is commonly used to study cancer biology [15]. Thus, we have focused on the comparative effects of butyrate and DCA on HCT116 colon cancer cell growth, cell cycle and apoptosis at 48–72 h in the present study. In contrast, we examined signaling molecules at early time points (e.g., 1.5 h) to limit the bystander effect.

2. Materials and methods

2.1. Cell cultures

HCT116 colorectal carcinoma cells were obtained from American Type Culture Collection and maintained in Dulbecco's modiied Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS; Sigma Chemical Corp., St. Louis, MO, USA). Sodium butyrate (NaB) and DCA were purchased from Sigma Chemical Corp. Stock cells were passaged twice weekly at ~<80% confluency [0.25% trypsin (Invitrogen), 1 mmol/l EDTA, in

Table 1
Effect of NaB and DCA on the cell cycle progression in HCT116 cells

Time	48 h				72 h			
NaB (mmol/l)								
NaB (mmol/l)	0	0.5	1	1.5	0	0.05	0.1	0.2
G1-phase cells (%)	34.2±1.8^{b}	32.4±4.4^{b}	41.5±5.4^{b}	53.6±6.2^{a}	60.2±3.4^{b}	64.4±0.6^{ab}	68.3±3.4^{ab}	64.9±4.2^{a}
S-phase cells (%)	52.1±0.8^{a}	46.9±2.8^{a}	35.6±5.4^{b}	8.1±7.0^{c}	28.7±4.0^{a}	19.8±1.8^{b}	12.9±5.8^{bc}	9.6±1.8^{c}
G2-phase cells (%)	13.7±1.8^{c}	20.7±5.4^{b}	22.4±0.4^{b}	38.2±1.6^{a}	11.1±0.6^{c}	15.8±1.4^{bc}	18.9±3.0^{b}	25.6±5.2^{a}
DCA (mmol/l)								
DCA (mmol/l)	0	0.05	0.1	0.2	0	0.05	0.1	0.2
G1-phase cells (%)	33.3±0.8^{c}	39.1±2.4^{bc}	47.2±3.4^{b}	74.2±7.8^{a}	61.0±2.8^{c}	61.1±2.4^{c}	66.5±0.8^{b}	76.9±2.6^{a}
S-phase cells (%)	52.2±1.8^{a}	48.7±1.4^{a}	42.8±2.0^{b}	9.6±9.2^{c}	27.2±4.2^{a}	28.4±3.2^{a}	23.4±2.4^{a}	8.5±4.8^{b}
G2-phase cells (%)	14.5±2.4^{a}	12.2±3.4^{a}	10.1±2.4^{a}	16.6±4.4^{a}	11.9±1.6^{a}	10.5±1.8^{a}	10.2±2.0^{a}	14.6±3.6^{a}

Values are means±S.D., n=4. Means at a time without a common letter differ, P<.05. The HCT116 cells treated with NaB (2 mmol/l) or DCA (0.3 mmol/l) were not suitable for cell cycle analysis due to a significant amount of apoptosis.

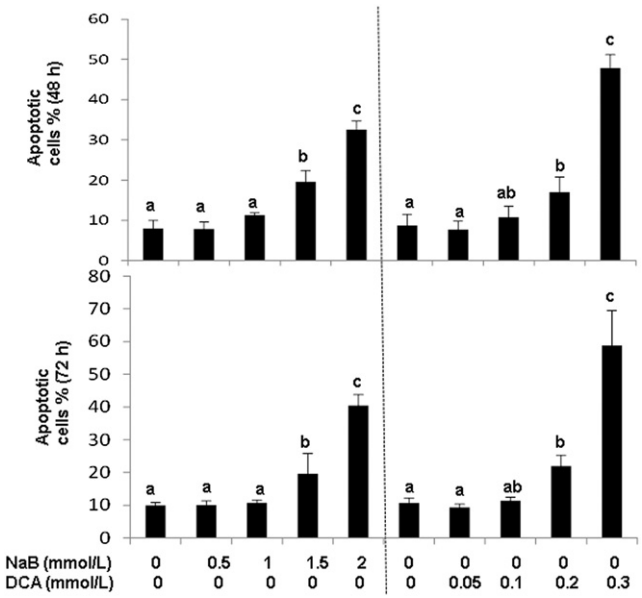

Fig. 2. Effect of NaB and DCA on HCT116 colon cell apoptosis for 48 and 72 h. Values are means±S.D., $n=4$. Means at a time without a common letter differ, $P<.05$.

Ca–Mg-free Hanks' balanced salt (Sigma), and viability was determined by trypan blue exclusion based on hemocytometer counts) and incubated in a humidified chamber at 36.5°C, 5% CO_2. Cultures were tested and found to be mycoplasma-free [18]. All butyrate- and DCA-treated cells (e.g., for cell cycle and apoptosis analyses) were grown and performed between passages 23 and 50 in standard media supplemented with 10% FBS.

2.2. Cell cycle analysis

Cell cycle was analyzed using flow cytometry with propidium iodide (PI) staining. HCT116 cells were trypsinized and washed once with phosphate-buffered saline (PBS) and fixed in 70% (vol/vol) ethanol at −20°C. After fixation, cells were washed with PBS and stained with 50 mg PI/L containing 6000 U RNase A/L. The DNA contents of the cells were determined by flow cytometry. Data were stored as list mode files of at least 10,000 single-cell events and analyzed by EPICS profile II and ModFit LT software (Coulter Corp., Miami, FL, USA, and Topsham, ME, USA).

2.3. Apoptosis analysis

Apoptosis was analyzed using a Guava Nexin Kit (Guava Technologies, Inc., Hayward, CA, USA). HCT116 cells were trypsinized and then suspended in growth media (DMEM with 10% FBS). In the apoptotic cells, molecules of phosphatidylserine (PS) are translocated to the outer surface of the cell membrane where Annexin V can readily bind them. Annexin V is a calcium-dependent phospholipid binding protein with high affinity for PS, a membrane component normally localized to the internal face of the cell membrane. At least 2000 single-cell events per sample were analyzed by the Guava PCA System.

2.4. Detection of intracellular reactive oxygen species production

2′,7′-Dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, OR, USA) is a nonfluorescent cell-permeable probe which is de-esterified intracellularly and rapidly oxidized to highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence reactive oxygen species (ROS) [19,20]. For each assay, HCT116 cells cultured on 24-well plates were preincubated with DCFH-DA (5 μmol/l) solubilized in dimethyl sulfoxide for 30 min at 37°C, washed three times with PBS, and the fluorescence signal of DCFH-DA (Ex=490 nm; Em=510 nm). The intracellular DCF was analyzed by a fluorescent microscope, and Image Pro Plus Version 6.2 software (North Central Instruments, Plymouth, MN, USA) was used for computerized quantification.

2.5. DNA breakage assay

DNA samples were then extracted by overnight incubation at 50–55°C in lysis buffer (50 mmol/l Tris–HCl, pH 8.0, 10 mmol/l EDTA, 150 mmol/l NaCl, 100 μg/ml proteinase K). These DNA samples were recovered by isopropanol precipitation, resuspended in Tris–EDTA–RNase (6 U/ml), analyzed on 1.9% agarose gels, and visualized by ethidium bromide staining. The intensity signals of genomic DNA fragmentation were analyzed by the UVP Bioimaging Systems (Upland, CA, USA).

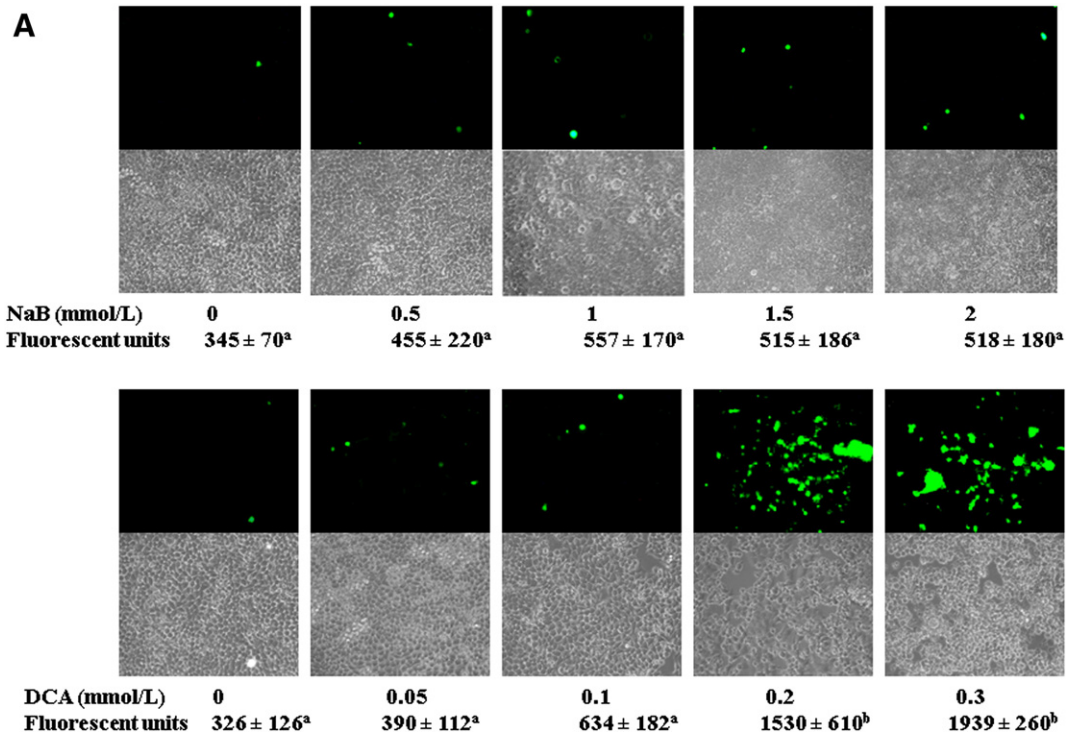

Fig. 3. Effect of NaB and DCA on the ROS fluorescent-signals and genomic DNA fragmentation. (A) A representative fluorescent photomicrograph of HCT116 cells stained by DCFH-DA probe showing intracellular ROS at 1.5 h. The area of intracellular ROS staining fluorescent signal was detected by a fluorescent microscope, and Image Pro Plus Version 6.2 software. (B) A representative DNA image showing the intensity of genomic DNA fragmentation at 1.5 h. The intensity signals of genomic DNA fragmentation were analyzed by the UVP Bioimaging Systems. Values are means±S.D., $n=4$. Means at a time without a common letter differ, $P<.05$.

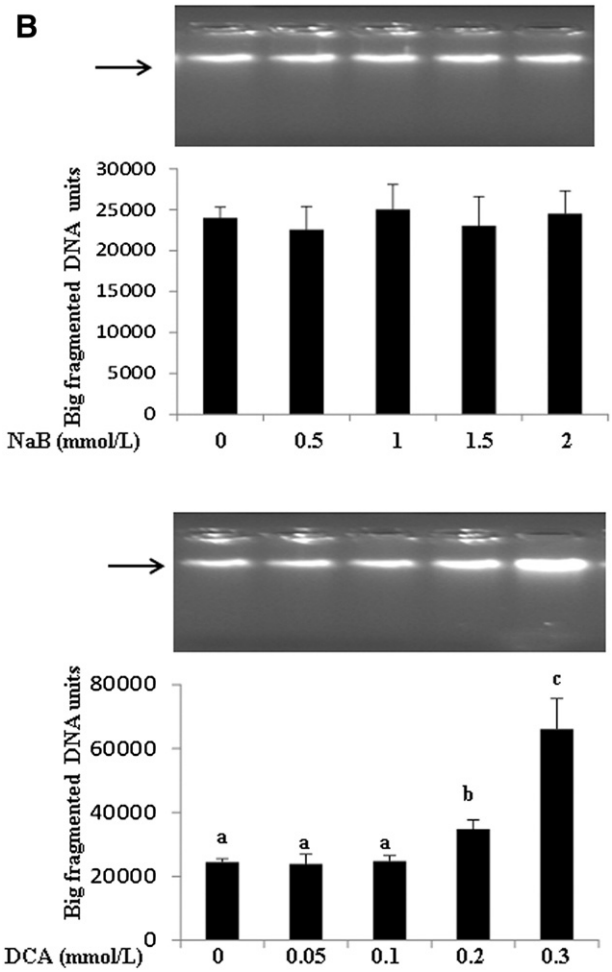

Fig. 3. (*continued*)

2.6. Western blotting analysis

After butyrate and DCA treatment for 1.5 or 15 h, adherent cells were scraped and pooled with the detached cells in 5-ml media, and then these cells were collected by centrifugation at 350×*g* for 10 min at 4°C; at least three independent experimental cell sample sets were collected. The cell pellet was washed once in ice-cold PBS and lysed in an assay buffer (Cell Signaling Technology, Inc., Danvers, MA, USA) with 1 mmol/l phenylmethylsulfonyl fluoride. After a brief sonication, the cell lysate was centrifuged at 14,000×*g* for 30 min at 4°C. The supernatant was designated as whole-cell protein extract and kept at −80°C. The protein concentration was quantified by the Bradford dye-binding assay (Bio-Rad laboratories, Richmond, CA, USA). Equal amounts of protein extract ~40 μg were resolved over 4%–20% Tris–glycine gradient gels under denaturing and reducing conditions and electroblotted onto PVDF membranes (Invitrogen). Membrane blots were blocked in PBS–0.05% Tween (vol/vol) supplemented with 1% (wt/vol) nonfat dry milk (BioRad, Hercules, CA, USA) at 4°C for overnight. Membranes were probed with antibodies against ERK1/2, caspase-3, PARP, Rb p21, and Bid antibodies and then incubated with an anti-mouse/rabbit (1:3000 dilution) horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in blocking solution for 1 h at RT. Blots were washed as above and proteins were detected by using an ECL plus kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) with the Molecular Dynamics ImageQuant system (Sunnyvale, CA).

2.7. Statistical analysis

Results are given as means±S.D.s. Data were analyzed using a one-way analysis of variance to compare the mean values, and Tukey–Kramer multiple comparison procedure was used for post hoc comparison of treatment means. JMP V9.0 (SAS Institute, Inc., Cary, NC, USA) was used for all statistical analyses, and differences with a *P*-value ≤.05 were considered statistically significant.

3. Results

3.1. Effects of butyrate (NaB) and DCA each on cell growth

The cell growth rate was inhibited by 16%, 34%, 60% and 80% for 48 h, and 21%, 39%, 72% and 89% for 72 h, respectively, in the cells treated with 0.5, 1, 1.5 or 2 mmol/l butyrate when compared with those untreated cells (Fig. 1). Similarly, the cell growth rate was inhibited by 11%, 32%, 64% and 83% for 48 h, and 12%, 46%, 77% and 92% for 72 h, respectively, in the cells treated with 0, 0.05, 0.1, 0.2 or 0.3 mmol/l DCA when compared with those untreated cells (Fig. 1).

3.2. Differential effects of butyrate (NaB) and DCA each on cell cycle progression

Butyrate and DCA increased G1-phase cell distribution and decreased S-phase cell distribution at 48, 72 h (Table 1). However, G2-phase cell distribution was increased by 0.6- and 1.8-fold for 48 h and 0.7 and 1.3-fold for 72 h, respectively, in the cells treated with 1.0 and 1.5 mmol/l butyrate but not DCA when compared with that of untreated cells (Table 1).

3.3. Effects of butyrate (NaB) and DCA each on apoptosis

Apoptotic cells were increased by 1.5- and 3.1-fold for 48 h and 1.0 and 3.1-fold for 72 h, respectively, in the cells treated with 1.5 or

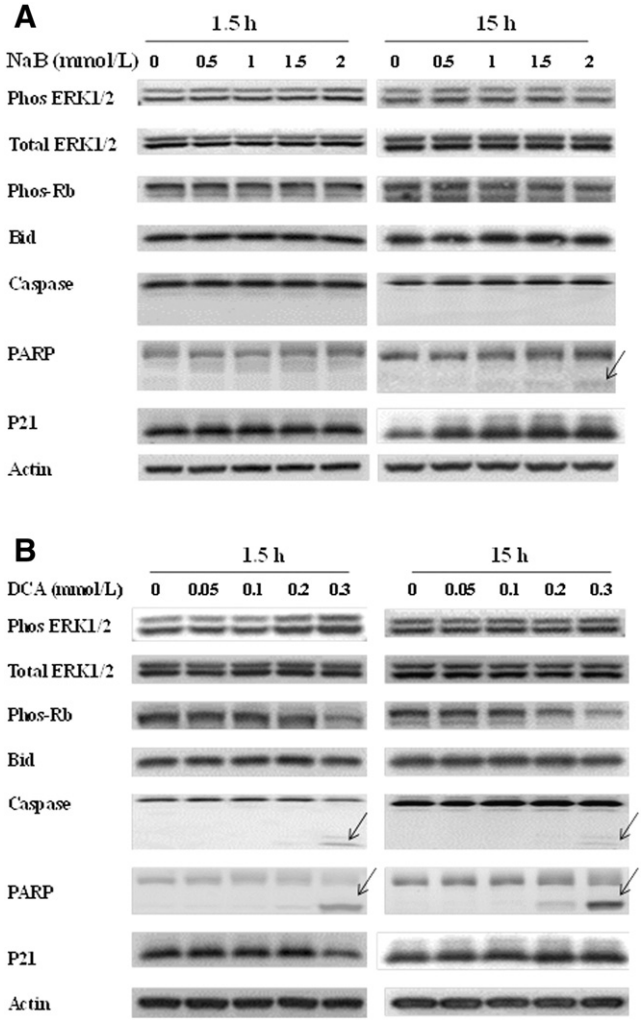

Fig. 4. Western blot analyses of the effects of NaB (A) and DCA (B) on intracellular signaling proteins in HCT116 colon cells for 1.5- or 15-h treatment. A representative Western blotting image was from three to five independent experiments.

2 mmol/l butyrate when compared with that of untreated cells (Fig. 2); Similarly, apoptotic cells were increased by 1.0 and 4.5-fold for 48 h and 1.1 and 4.5-fold for 72 h, respectively, in the cells treated with 0.2 or 0.3 mmol/l DCA when compared with that of untreated cells (Fig. 2).

3.4. Effects of butyrate (NaB) and DCA each on intracellular ROS production and DNA breakage

Intracellular ROS was readily detectable in the cells by DCFH-DA fluorescence assay in a dose-dependent manner after the addition of 0.05, 0.1, 0.2 or 0.3 mmol/l DCA for as little as 1.5 h (Fig. 3 A). However, little intracellular ROS signals were detected in the cells after the addition of 0.5, 1, 1.5, or 2 mmol/l butyrate (Fig. 3A). Similarly, the intensity of genomic DNA breakage, the cleavage at chromatin loop domains (50–300 kb), was increased in the cells treated with 0.2 or 0.3 mmol/l DCA (but not butyrate) for 1.5 h when compared with that of untreated cells (Fig. 3B).

3.5. Effects of butyrate (NaB) and DCA each on ERK1/2, caspase-3, PARP, Rb activation and p21 and Bid protein expression

To examine the cellular signaling molecules related to cell cycle arrest and apoptosis, we determined the phosphorylation status of ERK1/2 and Rb, activation of caspase 3 and PARP, and protein level of Bid and p21 when cells treated with butyrate or DCA for 1.5 or 15 h (Fig. 4; Tables 2 and 3). First, at 1.5-h time point, although the total ERK1/2 did not change, DCA but not butyrate increased the active phosphorylated ERK1/2 level by 1-fold when compared with those control cells (untreated cells). DCA but not butyrate decreased Rb phosphorylation and p21 expression by 35% and 30%, respectively, although the expression of apoptotic Bid gene expression did not differ. DCA but not butyrate activated caspase-3 and PARP enzymes, respectively (Fig. 4; Tables 2 and 3). Interestingly, at 15-h time point, DCA or butyrate treatment did not differ the expression of total, phosphorylated ERK1/2 and Bid, although the expression of apoptotic Bid gene expression did not differ. DCA activated caspase-3 and PARP enzymes, while butyrate had only very limited activation of PARP enzymes, respectively. DCA but not butyrate decreased the expression of Rb by 60%. However, butyrate but not DCA increased p21 protein level by 0.9-fold (Fig. 4; Tables 2 and 3).

4. Discussion

Butyrate, an intestinal microbiota metabolite of dietary fiber, has been shown to exhibit colon cancer-preventive effects through cell cycle arrest and apoptosis [21,22]. On the other hand, because of the consumption of high-fat diet, the increasing level of DCA in colon lumen is believed to promote colon cancer via modulation of cell cycle and apoptotic process [10,12,14]. Thus, the study of the comparative effects of butyrate and DCA at physiological concentrations [10] on cell proliferation and early signaling

Table 2
Effect of NaB on the expression of cell proliferation genes

NaB (mmol/l)	0	0.5	1	1.5	2
Intensity units of Western blot bands					
p-ERK1/2 (1.5 h)	3377±257	3571±410	3695±291	3859±266	4000±692
p-ERK1/2 (15 h)	2880±202	2963±620	2550±624	2439±886	2873±1088
p-Rb (1.5 h)	6064±491	5492±519	5199±923	5698±1208	5164±967
p-Rb (15 h)	5797±363	5768±978	4842±1419	4364±1426	4128±956
Bid (1.5 h)	9257±402	9120±1463	9004±2029	8459±1757	8076±1481
Bid (15 h)	9396±752	8802±1201	8890±1252	9115±1535	8353±1529
Cleaved PARP (15 h)	ND	ND	ND	ND	201±78[a]
P21 (1.5 h)	1323±106	1420±237	1390±225	1215±228	1255±13
P21 (15 h)	1052±90[c]	1645±43[b]	1949±104[a]	2092±118[a]	2038±82[a]
Actin (1.5 h)	3168±346	3188±612	3352±408	3407±324	3645±138
Actin (15 h)	3679±467	3569±566	3506±789	3423±497	3153±271

Values are means±S.D., $n=3$–5. No detectable signals (ND) were found in cleaved caspase-3 (1.5 or 15 h) and cleaved PARP (1.5 h); total ERK1/2 did not differ. Means at a time without a common letter differ, $P<.05$.

Table 3
Effect of DCA on the expression of cell proliferation genes

DCA (mmol/l)	0	0.05	0.1	0.2	0.3
Intensity units of Western blot bands					
p-ERK1/2 (1.5 h)	2517±406[b]	2666±295[b]	2850±381[b]	3576±629[ab]	5004±1301[a]
p-ERK1/2 (15 h)	3004±270	2513±792	2723±567	2463±945	2684±1051
p-Rb (1.5 h)	5051±744[a]	4507±143[a]	4483±382[a]	4389±400[a]	3257±420[b]
p-Rb (15 h)	5477±431[a]	5587±600[a]	5417±588[a]	3647±302[b]	2175±181[c]
Bid (1.5 h)	9971±1313	9564±1221	10233±1293	10053±1512	8554±2070
Bid (15 h)	7965±670	8122±783	7992±1141	7299±2075	6530±1450
Cleaved caspase3 (1.5 h)	ND	ND	ND	ND	1762±714[a]
Cleaved caspase3 (15 h)	ND	ND	ND	ND	537±303[a]
Cleaved PARP (1.5 h)	ND	ND	ND	162±139[a]	1503±253[b]
Cleaved PARP (15 h)	ND	ND	ND	500±464[a]	1628±224[b]
P21 (1.5 h)	1229±58[a]	1322±88[a]	1326±107[a]	1295±193[a]	862±254[b]
P21 (15 h)	1034±186	1136±67	1150±160	1296±195	1345±246
Actin (1.5 h)	3186±285	3593±332	3610±567	3302±89	3343±129
Actin (15 h)	3229±92	3340±214	3058±116	3149±438	3221±426

Values are means±S.D., n=3–5. No detectable signals (ND), and total ERK1/2 did not differ. Means at a time without a common letter differ, P<.05.

pathways will further our understanding of the functional roles of a high-fiber vs high-fat diet in colon cancer risk.

It has been reported that butyrate at mmol/l and DCA at sub-mmol/l levels in the colon is well within physiological levels [4,5,10]. Our present data showed that the effect of 0, 0.5, 1, 1.5 or 2 mmol/l of butyrate on HCT116 cell growth inhibition was virtually identical to that of 0, 0.05, 0.1, 0.2 or 0.3 mmol/l DCA (Fig. 1). However, DCA and butyrate employed several distinct molecular pathways to modulate HCT116 cell proliferation. For example, although both butyrate and DCA increased the G1 cell fraction and decreased S-phase cell fractions, butyrate but not DCA increased the G2 cell fraction (Table 1). In addition, DCA showed a stronger potential to induce apoptosis than that of butyrate (Fig. 2). These observations suggest that there are different molecular targets through which butyrate and DCA inhibit cell proliferation. It has been well recognized that ROS play an important role in DNA-damage response and ERK1/2 up-regulation-related apoptosis [23–25]. We found that DCA but not butyrate rapidly increased the cellular fluorescence signal of DCF [19,20] (Fig. 3A), suggesting an increase in cellular ROS level in HCT116 cells, which may relate to the difference of other subsequent molecular targets. Similarly, DCA but not butyrate increased high-molecular-mass DNA fragmentation in as little as 1.5 h (Fig. 3B). This finding is consistent with the report that during apoptosis, the cleavage at chromatin loop domains (50–300 kb) to generate high-relative-molecular-mass DNA fragments is the initial DNA breakage [26,27], and it is also in agreement with the observation that DCA showed a stronger potential of apoptotic induction than that of butyrate (Fig. 2).

To gain further insights into cell cycle and apoptosis, we examined several related signaling proteins (Fig. 4; Tables 2 and 3). The retinoblastoma protein (Rb) and ERK1/2 are essential regulators of cellular processes involved in carcinogenesis including cell proliferation and apoptosis [28,29]. It is known that activation of phosphorylating ERK1/2 regulates tumor cell proliferation, and inhibition of ERK1/2 suppresses the growth of colon tumors [30]. On the other hand, Rb governs cell cycle progression and acts as a key tumor suppressor when it is activated/phosphorylated [28]. In the present study, DCA but not butyrate showed a stronger potential to induce phosphorylation of ERK1/2 and de-phosphorylation of Rb, respectively. This observation suggested that DCA enhanced the tumor-promoting (ERK1/2) pathway and inhibited the activation of tumor (Rb) suppressors. Second, PARP, a 116-kDa nuclear chromatin associated enzyme, a critical executioner of apoptosis, is responsible for the proteolytic cleavage of many key proteins including activation of caspase-3 which requires proteolytic processing [31]. Our data demonstrated that the activation of caspase-3 and PARP occurred in those cells treated with DCA, but not butyrate, in as little as 1.5 h. This observation may be directly related to the DCA-induced cellular ROS levels which is a powerful promoter to induce apoptosis/genomic DNA breakage [27,32,33]. Third, Bid, a pro-death Bcl-2 family protein, connects the death receptor and mitochondrial apoptosis pathways [34]. The cyclin-dependent kinase inhibitor p21 (WAF1/CIP1) is a key mediator of p53-dependent cell cycle arrest and may play the role of a tumor suppressor in cancer [35]. That both DCA and butyrate did not affect Bid protein level suggests that the death receptor pathway plays little role in inhibiting HCT116 cell proliferation in the current experimental setting. Interestingly, that butyrate but not DCA dramatically increased the p21 expression suggests that butyrate may exert its anticancer property via p53/p21 cell cycle arrest pathway [35]. These results demonstrate that several molecular targets of butyrate and DCA are distinct. The most intriguing finding is that butyrate increased the tumor suppressor (e.g., p21) protein expression, while DCA increased tumor-promoting (ERK1/2) pathway and decreased tumor suppressor (Rb) activation [36,37]. These data, in part, reveal the underlying anticancer action of butyrate and the tumor-promoting effect of DCA [7,10,12]. It is believed that certain subpopulation of cells which are resistant to DCA-induced cell cycle arrest/apoptosis accumulates mutations and develops tumor cells [10,17,38]. With several distinct molecular pathways to inhibit colon cancer cell proliferation, it is conceivable that butyrate may inhibit the growth DCA-resistant subpopulation of cells *in vivo*. These findings provide the molecular basis that a butyrate-produced diet (e.g., high-fiber intake) may greatly reduce the cancer-promoting effect of high-fat meals in the human gut.

Taken together, butyrate and DCA play common and distinct roles in colon cell cycle/apoptotic process, and butyrate increased the tumor suppressor (e.g., p21) protein expression, while DCA increased tumor-promoting (ERK1/2) signal/pathway and decreased tumor suppressor (Rb) activation.

Acknowledgments

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